MATERIALS AND METHODS

The experiments were performed on adult male rats (Wistar strain), obtained from the National Laboratory Animal Center, Salaya Campus, Mahidol University. The initial weights of the animals were about 250-280 g. All animals were housed in the animal room where the temperature was maintained approximately at 25° C with 12hr dark - light cycle and provided with chow and water ad libitum. Prior to each experiment, the animals were allowed to adapt to the new environment for at least 5 days. The animals were then randomly assigned to one of the following four experimental groups (10 rats per group), (Figure 1).

Group I : Control group

Group II : Hypothyroid group

Group III : Exercise group

Group IV : Hypothyroid - exercise group .

Hypothyroidism (groups II and IV) was induced, according to the modified method of Fitzsimons, et al. (1990), by intraperitoneal injection of 1.2 ml/kg body weight of 1% Propylthiouracil (PTU, Sigma Chemical Co., St. Louis, USA) solution every other day for 12 weeks. Instead of PTU injection, the control animals (groups I and III) received intraperitoneal injection of normal saline solution (NSS) at the volume of 1.2 ml/kg body weight every other day throughout the experimental period.

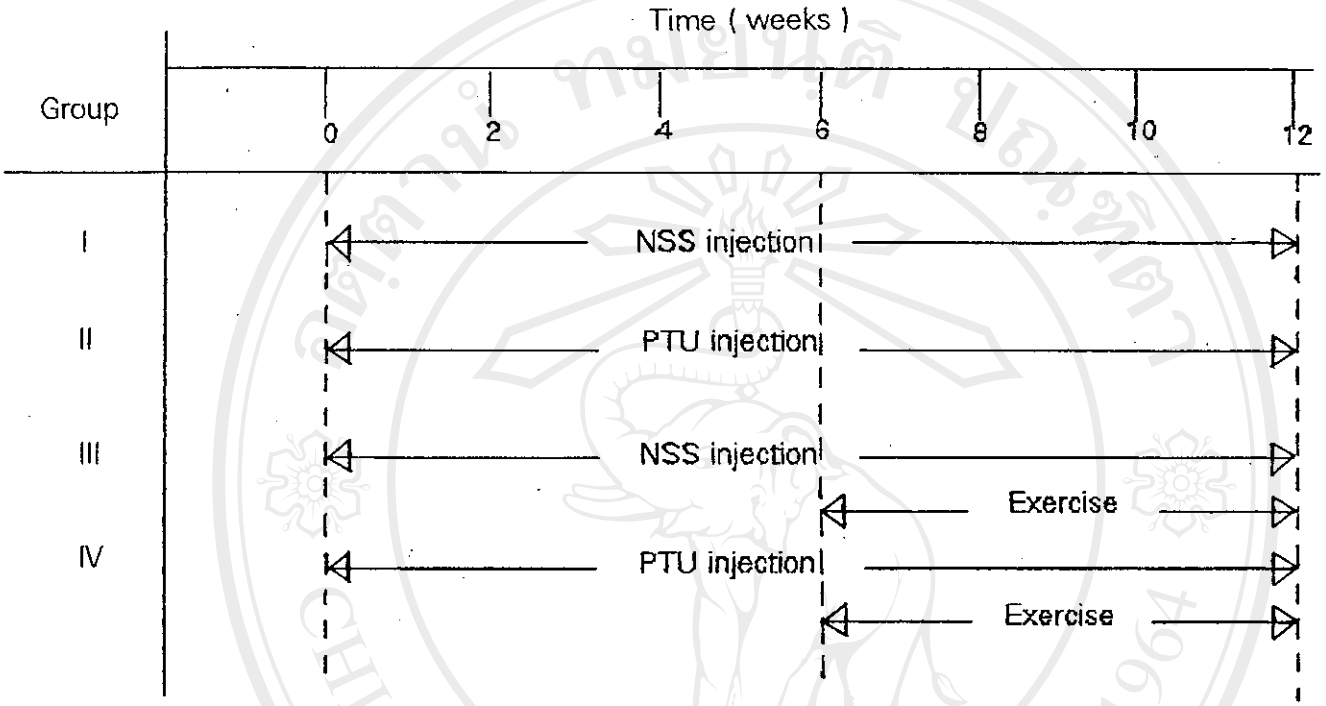

Figure 1 Diagrammatic illustration of the classification of the experimental groups.

Exercise Protocol

Exercise training program which was modified from the training protocol of Rupp, et al. (1984) was used. The training was 6 weeks in duration, beginning from the 7th week until the 12th week of the experimental period. Firstly, the animals (groups III and IV) were allowed to perform swimming in the water bath with density of 30 rats/m^2 surface area. The temperature of water in the bath was maintained at about 35°C and the water level was about 40 cm high, in order that the lower limbs and tails of animals did not attach to the bottom of the bath. The duration of swimming was 10 minutes at the beginning and was increased daily by 10 minutes step. At the 10th day, the total duration was 100 minutes, swimming exercise was then separated into two successive sessions of 50 minutes duration, each at intervals of 10 minutes rest. The duration of swimming was daily increased by 10 minutes step for each session until the 14th day of the training period, the maximum duration of swimming for each session reached upto 90 minutes. After that, the animals were subjected to swim at this maximum duration twice a day until the 42th day of the training period.

Measurement of Growth Rate

The body weight and body length were measured every two weeks during the experimental period in an attempt to assess the growth rate of animals. The body length was estimated by the Bernardis's method. Briefly, the animal was anesthetized with ether and subsequently placed on a plexiglass

board. The length from nose to origin of tail was measured by a tapeline. The relative obesity of animal was determined as obesity index which was calculated from the following equation (Bernardis and Skelton, 1967) :

$$\text{Obesity index} = \frac{\sqrt[3]{\text{body weight (g)}} \times 10}{\text{naso - anal length (mm)}}$$

Collection of Blood Sample

The blood samples were collected every 4 weeks by the following procedure. The animal was anesthetized with ether. A small incision about 1 - 1.5 cm length was made anteriorly through the skin on the neck. The surrounding connective tissue was cleared and the external jugular vein was exposed. Then, 1 ml of venous blood was withdrawn and the incision was sutured to prevent evaporative fluid loss. The blood sample was taken into a glass test tube. After standing at the room temperature for 2 hrs, the blood sample was centrifuged for 10 min at 2000 rpm in a Centrifuge (H - 103 N series, Kokusan Ensinki Co., Ltd., Japan) to separate serum from the cellular constituents. All serum samples were stored in appropriately marked centrifuge eppendrop at about - 20°C until time for assay.

Biochemical Analysis of Serum Thyroid Hormone Levels :

Determination of total serum thyroxine (T_4)

Radioimmunoassay (RIA) technique was used for the biochemical analysis of total serum thyroxine (T_4). The double antibody canine T_4 kits were purchased

from Diagnostic Product Corporation, USA. The principle of T_4 RIA was generally known that T_4 in the serum sample competed with radiolabeled (T_4) (^{125}I - T_4) to bind on T_4 - antibody. At optimal incubation time and temperature, the T_4 bound to its antibody at equilibrium stage. Then, the antigen - antibody complexes were separated from free T_4 by double antibody with polyethyleneglycol method. After centrifugation at 1500 g for 30 minutes, the precipitances of bound fraction was sedimented at the bottom of test tube. These precipitances of all tubes were counted for radioactivity in gamma counter (Model 1277, LKB Wallac, Finland). The results of radioactivity from precipitances of T_4 calibrators were plotted against T_4 concentration in logit - log graph paper to construct a standard curve. Hence the T_4 concentration in each unknown sample was read out by interpolation of each radioactivity counts on standard curve.

Determination of total serum triiodothyronine (T_3)

Total serum triiodothyronine (T_3) was assayed by RIA (Coat - A - Count Canine T_3) which was purchased from Diagnostic Product Corporation, USA.

The principle of T_3 RIA, Coat - A - Count procedure, was generally known that T_3 in the serum sample competed for a fixed time with radiolabeled T_3 (^{125}I - T_3) to bind on T_3 - antibody that was immobilized to the wall of a polypropylene tube. At optimal incubation time and temperature, the antigen - antibody complexes were separated from free T_3 by simply decanting the mixture solution. Then, the tube was counted for radioactivity in gamma counter. The results of radioactivity from the tube of T_3 calibrators were plotted against T_3 concentrations in logit - log graph paper to construct a standard curve. Hence

the T_3 concentration in each unknown sample was read out by interpolation of each radioactivity counts on standard curve.

Preparation of Muscles for Neurally - evoked Contraction in situ.

The animal was anesthetized with pentobarbital sodium, 50 mg/kg body weight, by intraperitoneal injection and given the maintenance doses as needed. Tracheotomy was performed and the trachea was cannulated immediately with a polyethylene cannula (internal diameter = 2 mm) to ensure the patent airway.

The hindlimb musculatures of both left and right sides were exposed by making a midsagittal incision running from the calcaneus to the midportion of the biceps femoris. By a blunt dissection technique, the skin was freed from the underlying musculature. The connective tissue lying on the exposed area was removed. The biceps femoris was isolated and detached from its insertion along the lateral border of the tibia. Then, isolation of soleus was done on the left side of hindlimb whereas isolation of plantaris was carried out on the right side. The operation of muscle, leaving the blood and nerve intact, was carried out according to the method outlined by Caiozzo, et al. (1991) as follows. The distal tendon of the soleus or plantaris was isolated and detached from its insertion along the Achilles tendon. Then, the adjacent musculature and surrounding connective tissue were removed, in order that the isolated soleus or plantaris had unrestricted movement.

Once both left soleus and right plantaris were completely isolated, the animal was subsequently placed in supine position on a plexiglass board. The sciatic nerves of both left and right sides were exposed by making a skin

incision of a mid portion of the posterior aspect of the thighs. The surrounding connective tissue was removed and the sciatic nerve was cleared about 1 cm long. The proximal part of the nerve was tied tightly by a thread and the nerve was cut at a point above the ligature in order to avoid any central connection. A distal part of nerve was placed on a pair of stainless steel bipolar stimulating electrode connected to an electronic stimulator (Model S88, Grass Instruments Co., Quincy, Mass., USA). A metal bar was, then, inserted through the left and right sides of the knee joints and another metal bar was pierced through both sides of ankle joints. The ends of the metal bars were attached to the poles besides the plexiglass board in order to immobilize the lower limbs. Finally, the distal tendon of the soleus or plantaris was cut, tied with a strong thread and attached to a force - displacement transducer (FT 03 C, Grass Instrument Co., Quincy, Mass., USA) for monitoring contractile properties on a polygraph (Model 7, Grass Instrument Co., Quincy, Mass., USA). Throughout the experimental period,muscle temperature was controlled by soaking the muscle with the dripping warmed liquid paraffin which was kept at a temperature of 30 - 30.5°C by using radiant heat from a lamp.

A schematic representation of muscle preparation for studying neurally - evoked contraction was illustrated in Figure 2.

Measurement of the Contractile Properties

The animal was left at the room temperature for 30 min to stabilize condition prior to the measurement of the contractile properties. The isolated

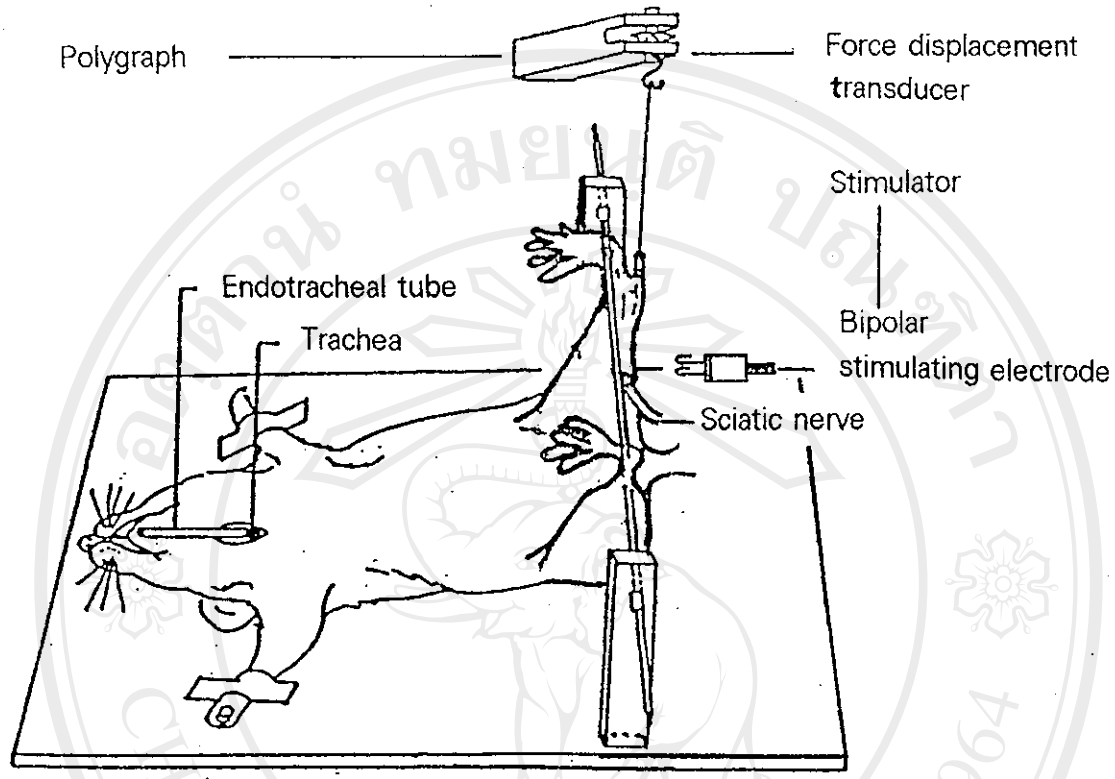

Figure 2 A schematic representation of the muscle preparation for studying neurally - evoked contraction.

muscle was, then, adjusted according to the method of Close (1964) to its optimal length at which the active tension was maximal. The contractile properties of both left soleus and right plantaris were assessed by the following procedures :

I. Determination of the maximal voltage of the muscle.

The maximal voltage of the muscle was assessed by stimulating the sciatic nerve with a single rectangular pulse of 0.2 ms duration. The intensity of stimulating current was about 50 volts at the beginning and increased in 1 volt steps until the maximal voltage, at which active tension approached the maximum, was obtained.

II. Measurement of the twitch characteristics.

The muscle was stimulated via the sciatic nerve by a single stimulation at the supramaximal voltage with 0.2 ms duration. The isometric twitch elicited by a single supramaximal stimulation was recorded on the polygraph at paper speed of 100 mm/sec. The isometric twitch tension (P_T), time to peak tension (TPT) and one - half relaxation time ($RT^1/_2$) was measured by vernia calipers from the myogram of single twitch (Figure 3).

III. Determination of force - frequency relationships.

The force - frequency relationships were assessed by the modification technique of Caiozzo, et al. (1991). The muscle was stimulated via the sciatic nerve by repetitive supramaximal voltage with 0.2 ms duration at the stimulation frequencies of 5, 10, 15, 20, 30, 40, 50,75, 100, and 125 Hz, respectively. The train durations of the soleus and plantaris were 1000 and 500 ms, respectively, and the train rate was one per second. Stimulation of the muscle was stopped after the isometric tetanic tension produced in response to each stimulating frequency became steady and the muscle was, then, allowed to

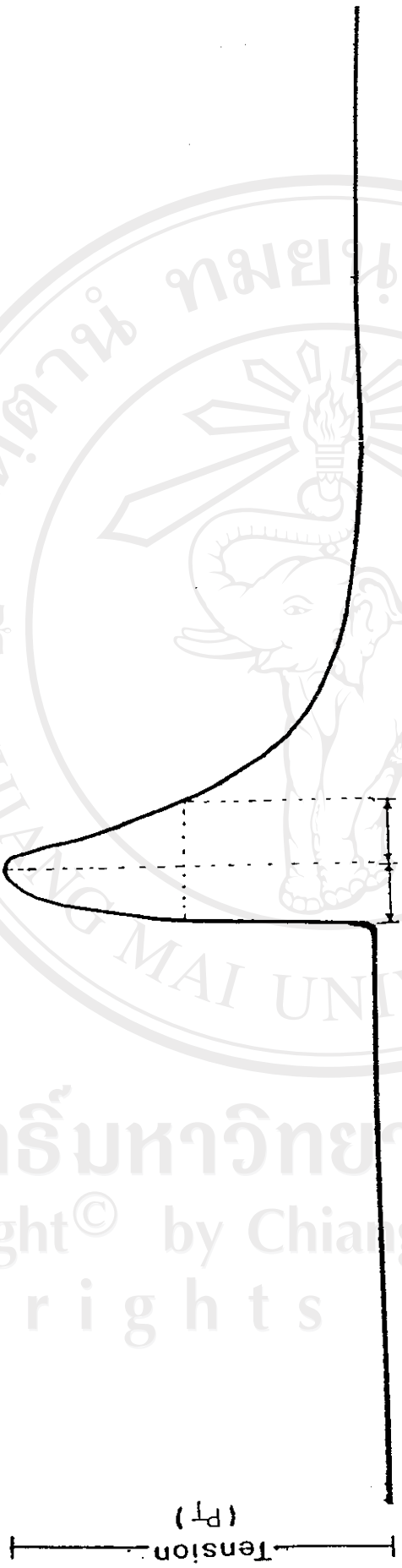

Figure 3 Determination of time to peak tension (TPT), one - half relaxation time (RT1/2) and muscle tension (P_T), from a isometric single twitch of skeletal muscle.

rest for 1 min interval. The isometric tetanic tensions elicited by repetitive supramaximal stimulation at each frequency were recorded on the polygraph at paper speed of 25 mm/min.

Determination of Muscle Endurance Properties

Endurance properties of the muscles were determined by the method of Burke, et al., (1971).

After 30 min of the completion of the measurement of force - frequency relationships, the fatigability of the muscle was assessed by stimulating the sciatic nerve with supramaximal voltage at a stimulation frequency of 40 Hz, train duration of 330 ms at a rate of 1 train/s, for a total 4 min. The isometric tetanic tension was recorded on the polygraph at paper speed of 25 mm/min.

Muscle endurance properties was expressed as the fatigue index (Burke, et al., 1971; Van Lunteren, et al., 1990) which was calculated from the following equation :

$$\text{Fatigue index} = \frac{\text{the force at the end of 2 min}}{\text{the initial force of contraction}}$$

Measurement of Muscle Weight

At the end of the experiment, both the soleus and the plantaris were excised from the animal. The muscles were stripped off fat and connective tissue, blotted dry and weighed on an electronic balance (Model R 300 S, Sartorius Research, Japan).

Statistical Analysis

Data were recorded as means ± SE. Statistical comparisons between groups as well as the changes from control to experimental period in each group were analyzed by using Student 's t test. $P < 0.05$ was considered to be significant difference.